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RHABDOMYOSARCOMA IN THE CHICKEN

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A case of a hen's-egg-sized rhabdomyosarcoma which originated in the skeletal muscles (*M. biceps femoris*) in the right leg was described in a 140 day female chicken. A broad-bean-sized metastatic nodule was seen in the spleen. Histologically, the tumor cells showed pleomorphism and definite cross striations were found only in some tumor cells. The ultrastructure revealed bundles of myofilaments and electron opaque substance resembling the components of Z-lines in some part of the cytoplasm of the tumor cells. These findings are suggestive of a striated muscle cell origin of the tumor.

There were numerous C-type virus particles in the intercellular spaces of the tumor.

INTRODUCTION

Reports of rhabdomyoma or rhabdomyosarcoma of striated muscle origin are rare in all domestic animals. Rhabdomyoma from myocardium has been relatively well-known in swine^{2,4,7~9,13,18,20} and guinea pigs^{10,13,21}. Rhabdomyomatous tumors appearing in the skeletal muscles occur particularly in the limbs²³, but some have been in the lungs³. According to HADLOW and KAST & HÄNICHE, malignant tumors of skeletal muscle origin have been reported only twice in horse, 3 times in cattle, twice in sheep, twice in dog and once each in mouse, deer and chamois (table 1). Already reported cases of rhabdomyoma or rhabdomyosarcoma in chickens were listed as table 2. Rhabdomyosarcoma in chickens only took place in 3 out of the 8 cases listed above. The present paper report another case of rhabdomyosarcoma in chicken.

MATERIALS AND METHODS

The tissues were fixed in 10% formalin and embedded in paraffin. Sections were routinely stained with hematoxylin and eosin. Special stains used on selected sections included WEIGERT's iron hematoxylin and eosin, AZAN stain VAN-GIESON stain and periodic acid-SCHIFF (PAS) reaction.

Materials for electron microscopy were fixed in Millonig's phosphate-buffered osmium tetroxide solution and dehydrated with a graded series of ethanol solutions. The tissues were embedded in a plastic mixture¹⁶. Sections were made with a Porter-Blum MT-1 ultramicrotome, stained with uranyl acetate and REYNOLD's lead citrate, and examined with a JEM type 7 electron microscope.

RESULTS

Clinical history

The present case was a 140 day female chicken of the White Leghorn variety (Heisdorf Nelson). She was one of the affected chickens which were sent from one poultry farm in Naganuma, Hokkaido to our laboratory for diagnosis. Most of these chickens were diagnosed as having a classical form of MAREK's disease. She was vaccinated twice at 35 and 81 days against Newcastle disease and also vaccinated at 35 days against fowl pox disease. She showed normal nutritional condition, but she stretched both her legs and was unable to stand up. There were no remarkable findings, except these symptoms. She was killed by exsanguination.

According to the owner, 5 cases of similar muscle tumors have been encountered in his farm during the past year. This farm was keeping about 32,000 birds at that time.

Gross Pathology

A hen's-egg-sized tumor mass was found in the region of the biceps femoris of the right leg. The tumor mass was somewhat elastic and firm. The tumor was demarcated from the adjacent muscles and surrounded with an ill-defined thin fibrous capsule. On the cut section, the tumor parenchyma was yellowish white and firm. Irregular strands of texture with congestion were seen with the naked eye. The sciatic nerve ran through the central part of the tumor.

The size of the spleen was as big as the head of the little finger. A broad-bean-sized tumor nodule was adjacent to the spleen. The spleen showed atrophy with an irregular outline due to the pressure of the tumor growth. The gross character of the tumor was similar to that of the tumor of the leg. There were no remarkable lesions except the tumors.

Histopathology

Primary lesion The tumor lesion was generally well demarcated from the adjacent normal tissues, but in some part the lesion was poorly defined, and transition from normal muscle cells to tumor cells was seen. There were perivascular lymphocytic accumulations with small numbers of heterophils as an inflammatory reaction principally at the peripheral area of the tumor and, to a lesser extent, in the central area of the tumor.

The tumor exhibited irregular whorls or an interlacing appearance of bundles of cells and fibers. The central part of the tumor showed irregular necrotic foci, congestion and hemorrhages, and showed cystic degeneration. These areas showed a loosening appearance and consisted of spindle-shaped cells. These cells contained a large number of fine vesicles or large vacuoles in the cytoplasm. AZAN stain showed sparse thin strands of collagen fibers that made the supporting fine network of the tumor, but in some area these fibers showed marked proliferation.

The tumor was composed of extremely pleomorphic cells, and large or small spindle-shaped or strap-shaped cells with cytoplasmic longitudinal fibrils were prominent. In the anaplastic area of the tumor, rounded cells with rather abundant cytoplasm and a single large nucleus, Purkinje-like cells with pale cytoplasm and hyperchromatic gigantic single pale nucleus or binucleated cells were intermingled (fig. 3). Multinucleated giant cells of

bizarre shapes were often found and they had sometimes more than ten nuclei (fig. 7). These cells had strongly acidophilic cytoplasm varying from a homogeneous to a granular appearance and had often numerous fine vesicles in the cytoplasm. On the other hand, in the less anaplastic area of the tumor, long spindle-shaped cells were prominent and arranged as bundles. These findings were apparently suggestive of fibrosarcoma (figs. 5 & 6). Among these various tumor cells, there were numerous vacuolated cells (fig. 4). They had an appearance similar to so-called "spider cells." There were PAS-positive granules in the cytoplasm of some of the pale and non-vacuolated cells.

The tumor tissues had a complex network of blood vessels. In carefully examined sections stained with iron hematoxylin and eosin, the long strap-shaped cells with abundant cytoplasm usually had longitudinal fibrils and sometimes cross striations were found (fig. 6).

Hyaline degeneration of the muscle fibers was occasionally found in the muscle tissues adjacent to the tumor. Muscle fibers in the transitional area between normal and tumor tissues (fig. 1) were shown in small size and the interstitium showed loosening appearance. Muscle cells showed gradually loss of striation from normal to tumor cells. On rare occasions, mitosis of the tumor cell with cross striation was detected (fig. 2).

Metastatic nodule in the spleen The tumor mass was demarcated sharply from the spleen. The tumor cells showed an irregular arrangement with bundles and the central portion showed irregular arborescent necrotic foci. The component cells of the tumor were similar to those of the primary lesion and consisted of principally spindle-shaped or strap-shaped cells. There were numerous vacuolated cells which seemed to be so-called "spider cells." Mitosis was frequently observed. Although longitudinal fibrils in the cytoplasm was easy to find out, cross striation could not be demonstrated.

Other lesions

Sciatic nerve At the portion of the nerve fiber passing through the tumor tissue, the axis-cylinder became granular and fragmented. The myelin sheaths underwent dissolution. Fibrosis was shown in some part of the interstitium of the nerve. Perivascular lymphocytic infiltration, edema, degeneration and loss of nerve fibers were observed in the other part of the nerve.

Skeletal muscles of the legs Atrophy of the muscle fibers. Prominent proliferation of the enlarged, vesicular muscle nuclei was conspicuous. There were no remarkable lesions in the other visceral organs and tissues.

Electron microscopy

Tumor cells The tumor was composed of cells of various shapes and sizes, but predominantly spindle-shaped cells. The typical fine structure of these cells was as follows: The nuclear margin was irregular with indentations, and nuclear pockets produced by cytoplasmic invagination into the nucleus were often observed. There was condensation of chromatin at the marginal area of the nuclear envelope, enlargement of the interchromatin spaces and a large nucleolus. The tumor cells had abundant cytoplasm with rich polyribosomes and well developed rough surfaced endoplasmic reticulum. Some bundles of myofilaments and electron opaque substances resembling components of Z-lines were found in one corner of the tumor cell. At the cell boundaries between two cells, there were desmosome

TABLE 1 *Rhabdomyoma and rhabdomyosarcoma in animals*

AUTHOR		RHABDOMYOMA	RHABDOMYO-SARCOMA	SPECIES
HIERONYMI & KUKLA	1921	1		
STENSTRÖM ¹³⁾	1921	1		
JOEST ¹³⁾	1923	1		
HENNEBERG	1928	1		
CLAUSSEN	1938	1		
NORDLUND	1940	1		swine
FØLGER ⁷⁾	1951	1		
TESTI ⁷⁾	1956	1		
ENGLERT	1959	1		
HJÄRRE ¹³⁾	1962	1		
KAST & HÄNICHEN	1967	3		
OMAR	1969	1		
BOUCK	1906	1		
MACCHIONI ¹³⁾	1936	1		
PIRES & MUCCILO	1939	1		
FONTENAILLE <i>et al.</i> ⁷⁾	1952/53		1	cattle
SALCUNI ⁷⁾	1953		1	
LABIE & HOVASSE ¹³⁾	1955		1	
JABB & KENNEDY	1963	1		
FELDMAN ⁷⁾	1932		1	horse
MÜLLER	1953		1	
DAY	1922	1		
COLLET <i>et al.</i> ¹³⁾	1952		1	sheep
ANDERSON ⁷⁾	1957		1	
BISBOCCI ¹³⁾	1942	1		
COLLET & TISSEUR ⁷⁾	1949		1	dog
WORLEY & GORHAM	1954		1	
BÜRGISSER ⁷⁾	1958		1	deer
BÜRGISSER ⁷⁾	1958		1	chamois
HUEPER	1941	1		
WEBER ¹³⁾	1949	1		guinea pig
ROONEY	1961	1		
HURLEY	1956		1	mouse

TABLE 2 *Rhabdomyoma and rhabdomyosarcoma in chickens*

CASE NO.	SEX	AGE	SITE	DIAGNOSIS	AUTHOR	
1	♀		Skeletal muscles of the sternum multiple, 6 separate $1\frac{1}{2} \times 1 \times \frac{1}{4}$ cm	Fibromyoma striocellulare	MEYER	1922
2	♂		Skeletal muscles, region of the hip joint	Transplantable rhabdomyoblastoma	PEYRON & BLIER ⁵⁾	1927
3	?		Pectoral muscle submental region	Rhabdomyoma	BABIC ⁵⁾	1931
4	♂	8 M	The flexor, extensor muscles, in both thighs, pectoral, thorax muscles, multiple tumors	"	OLSON & BULLIS	1942
5	♀	10 M	The semitendinosus muscle with secondary nodule on the intestine small tumor $20 \times 15 \times 7$ mm	"	"	
6	♀		The right extensor muscles of the knee a walnut-sized, lungs, myocardium hemp-seed sized	Rhabdomyosarcoma	KROGH	1953
7	♂		The left flexor and extensor muscles walnut-sized hen's-egg-sized lungs, pea-sized	"	"	
8	♂		Heart 25×12 mm	"	GRICE et al.	1957

structures (fig. 8). These findings were observed in both the primary tumor and in the metastatic nodule in the spleen.

In the intercellular spaces of the tumor, there were numerous particles similar in morphology to the C-type leukemia viruses. The size of these particles was ca. 100 m μ in diameter and they had a central nucleoid of ca. 40 m μ in diameter. They had numerous peripheral knobs on the outside of the particles (fig. 9).

DISCUSSION

Tumors of striated muscle origin have been classified as rhabdomyoma or rhabdomyosarcoma depending on whether they were regarded as benign or malignant, respectively. Distinction between the two is not always evident. Some workers used the term "rhabdomyoma" including both forms of the tumor. It is doubtful whether benign rhabdomyomatous tumors should be referred to as true neoplasm and most pathologists⁷⁾ consider this tumor to be a malformation

or hamartoma rather than a true neoplasm.

The so-called "congenital rhabdomyoma of the heart" was reported rarely in domestic animals, especially young swine^{2,4,7~9,13,18,20}) as well as in man. Only fourteen cases were reported in swine in the previous literature. Rhabdomyoma has been reported also in cattle, dogs, guinea pigs and chickens (tables 1 & 2). In the so-called "congenital rhabdomyoma of the heart", there was one of the cytoplasm from which fibrillar processes extend to the periphery of the cell to form the so-called "spider cells." They contained glycogen in their cytoplasm.

Rhabdomyosarcoma arising in the skeletal muscles is rare in domestic animals. It is considered as highly malignant, because it forms metastasis. Rhabdomyoma or rhabdomyosarcoma in chickens was principally found in the skeletal muscles and one case of myocardial origin was found only by GRICE *et al.* (table 2). Rhabdomyosarcoma of skeletal muscle origin in chickens showed metastatic nodules in the lungs and hearts¹⁴). In the anaplastic area of the present case, the tumor cells were extremely pleomorphic and consisted principally of spindle-shaped or strap-shaped cells. Large Purkinje-like cells, multinucleated giant cells and rounded cells were intermingled with the above described tumor cells. These cells had often cytoplasmic longitudinal fibrils and sometimes cytoplasm varying from a homogeneous to granular appearance. There were many vacuolated cells with the appearance of so-called "spider cells." But unfortunately in our present case the character of the contents of the vacuoles could not be clarified, because of inadequate fixative. Muscle fibers showed gradual loss of striation especially in the transitional area between normal and tumor tissues. On rare occasions mitosis of the tumor cell with cross striation was found. On the other hand, the ultrastructure of the tumor cells in both the primary tumor and metastatic nodule in the spleen showed cytoplasmic myofilaments and electron opaque substances resembling components of Z-lines. These cells resembled myoblasts. Therefore these findings were suggestive of a myogenic origin of the tumor.

We considered the present case of rhabdomyosarcoma to have originated from the voluntary muscle tissues, and could point some resemblance to the undifferentiated pleomorphic rhabdomyosarcoma in man from the view point of morphology.

The interpretation of whether the C-type virus particles which were found in the intercellular spaces of the tumor are only contaminants or an oncogenic factor to the present muscle tumor is still unknown.

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EXPLANATION OF PLATES

PLATE I

- Fig. 1 Mitosis (arrow) are seen in the acidophilic myogenic cells in the transitional area between normal and tumor tissues
H & E × 770
- Fig. 2 Mitosis (arrow) is seen in a cell with cross striations
H & E × 1,240
- Fig. 3 Note pleomorphic cells and a Purkinje-like cell accompanied by a gigantic single nucleus and abundant pale cytoplasm in the anaplastic area of the tumor
H & E × 770
- Fig. 4 There are numerous vacuolated cells. The arrow indicates a large cell with few vacuoles in the pale abundant cytoplasm
H & E × 375

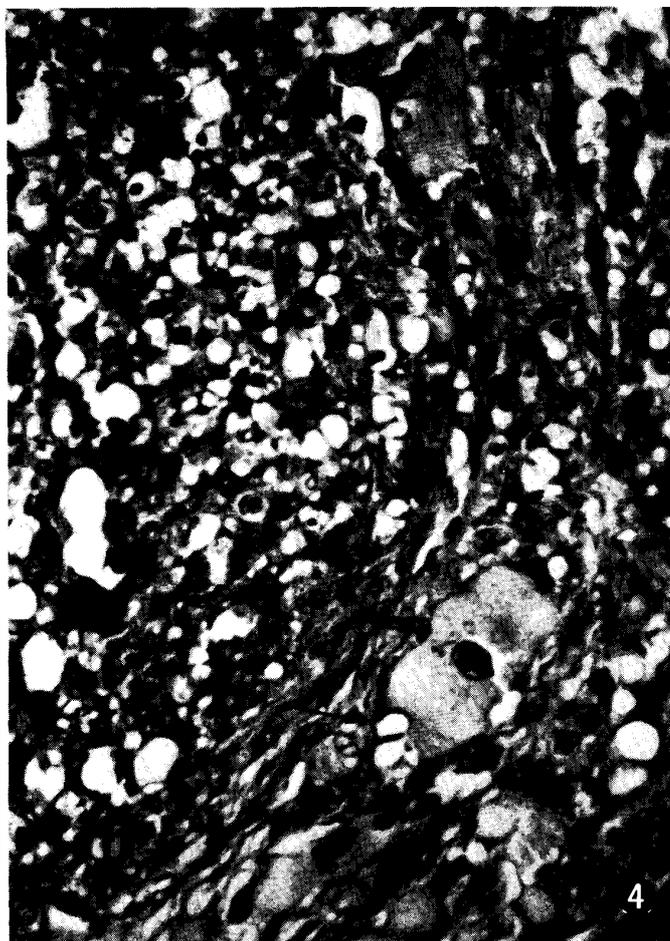


PLATE II

- Fig. 5 Interlacing appearance of spindle-shaped cells in the less anaplastic area of the tumor
H & E × 375
- Fig. 6 Higher magnification of fig. 5. Some of the tumor cells indicate evident transverse and longitudinal striations (arrow)
H & E × 770
- Fig. 7 Multinucleated giant cell with fine vesicles in the acidophilic cytoplasm
H & E × 770
- Fig. 8 In one corner of a myoblastic tumor cell, there are bundles of fine myofilaments and electron opaque substances resembling components of Z-lines (arrows ↙↘↙). C-type virus particle (↙) in the intercellular space and desmosome structures (↙↘) between two cells are also seen.
Electron micrograph from the tumor nodule in the spleen × 7,500
- Fig. 9 C-type virus particle × 60,000

